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GROWTH FACTORS AND POPULATION DENSITY IN THE AMERICAN COCKROACH, *PERIPLANETA* *AMERICANA*

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Abstract—Isolated cockroach larvae grow more slowly than larvae in groups, but the resultant adults are larger than those from groups of larvae. When single larvae are grown with adults, they grow as rapidly as larvae in groups, and the resultant adults are as large as those from isolates.

The following possible growth factors or conditions were examined: the effect of food, shelter, vision, aseptis, secretion from the tergites and cuticular exudates, the presence of a possible contact or air-borne pheromone, and physical contact as observed in ritualistic behaviour, which is here described for the first time; and certain other considerations. Neither air-borne nor contact growth pheromones could be demonstrated, nor could any significant effect by the exudates be shown. Food intake/unit weight of the isolated and grouped larvae did not differ significantly between the two categories. Physical contact with other larvae or with adults was the critical exteroceptive stimulus which promoted the rate of larval growth, but not the ultimate size. The greater size of adults derived from isolated larvae or from larvae reared with adults, as compared with those from grouped larvae, is attributed to ecdysone. Ecdysone, more concentrated in isolated than in grouped larvae, could also have a part in retarding the rate of growth of the isolated insect by reducing its locomotor activity and hence inducing a relatively lower metabolic rate.

INTRODUCTION

THE RATE of larval growth in terms of weight and development, and the weight of the resultant adults, of the American cockroach depend partly on population density, with an optimal level of density for each of these measures. It is especially notable that isolation at any stage of larval development causes the American cockroach to grow and develop more slowly than groups of larvae, except in very dense populations, and to produce adults of heavier weight. This influence of grouping is effective and reversible at any stage and therefore depends on a transitory factor(s) which varies with population density (WHARTON *et al.*, 1967). Observations on one or another of these aspects of growth have been made with the Oriental cockroach (LANDOWSKI, 1937), the German cockroach (PETTIT, 1940; CHAUVIN, 1946), the cricket (CHAUVIN, 1941), the desert locust (NORRIS, 1954; LÖHER, 1961), and many other insects and forms of life (ALLEE, 1934).

LÖHER (1961) has demonstrated that the factor which promotes maturity in the adult male desert locust is a pheromone which is transmitted from the mature males on contact or by air. CHAUVIN (1946) found that consumption of faeces could promote growth in the German cockroach, *Blattella germanica* (L.), but that incorporation of as much as ten per cent in the food was excessive and also failed to promote the growth of *Blaberus giganteus* (L.) and *Blatta orientalis* (L.); while MCFARLANE and HENNEBERRY (1965) have shown that contact with various fatty acids or their methyl esters could inhibit the growth of the cricket *Gryllodes sigillatus* (Walk.). Chauvin attributed the depressing effect of numbers on development of the German cockroach not only to a shortage (or, alternatively, a surplus) of space, but to antennal contact; whereas LEFKOVITCH (1964) attributed retardation of development of *Lasioderma serricorne* (F.) in restricted populations to a shortage of food rather than space.

When normal animals of originally equal weight are put on a standard diet and their weights come to differ, we can expect to find that the heavier animals have eaten more than the lighter. But food consumption reflects the state of well-being of the animal and is not necessarily itself a primary growth factor; it is but one criterion of the presence—or, more precisely, the efficacy—of growth factors in the metabolic cycle whose ultimate expression is growth. Since population density has profound consequences for the growth of the cockroach larva as expressed by differences in weight and maturation (WHARTON *et al.*, 1967), it is necessary to investigate the means by which these effects are brought about if we are to understand the nature of its influence on growth. Hence our present examination of the rôle of food consumption and other factors that may be involved in population density effects on growth.

MATERIALS AND METHODS

The American cockroach, *Periplaneta americana* (L.), was used in these investigations. The insects were generally kept in open, vaselined 6 × 8 in. battery jars in a dark incubator which was kept at a temperature of 29°C and ambient relative humidity, and lighted only for purposes of feeding and examination. Except where stated to the contrary, they were fed on Purina Laboratory Chow (PLC) and water and provided with a 3.25 × 3.25 in. (pint) Neptune Heavy Duty Liquid-tight Food Container as shelter. Special methods will be described in their appropriate sections. All deviations shown are standard deviations.

RESULTS

Food consumption

Newly hatched larvae were simultaneously distributed in jars singly and in groups of 2 up to 200 or more and then incubated. The rate of development and the weights of the larvae tended to increase as population density increased to an optimal level (WHARTON *et al.* 1967). Table 1 shows that by far the greatest percentage gain in weight is found between the 1's and 2's, followed by the 2's and 5's. The gain is slight and variable between the 5's and groups up to 60, but a

definite drop occurs with a density of 200. To determine the relationship of food consumption to growth and population density, groups of 20 and 200 larvae were compared throughout their period of development (Fig. 1, Table 2A). Food consumption was found to fluctuate with the moulting cycle and was at almost every weighing greater in the 20's than in the 200's, averaging 14.3 ± 12.6 and 10.5 ± 9.5 mg/roach per day for the 20's and 200's, respectively, during the first period of 13 weeks. Although the group of 20 consistently weighed more than the group of 200, there was no significant difference ($P = 0.3$, approx.) in the average amounts of food consumed/day per mg weight of larva, the amounts being

TABLE 1—GAIN IN WEIGHT (mg) PER INDIVIDUAL OF DIFFERENT POPULATION DENSITIES OF LARVAE

Pop. density	Days					Per cent increase or decrease
	22	42	69	85	91	
1	14	47	144	294	}	+ 97
2	19	75	266	580		
5	23	107	415	755		
10	22	93	341	680	}	- 17
15	23	116	441	769		
20	17	92	384	727		
25	21	115	413	755		
30	20	109	389	709		
60	21	120	450	723		
200				604	614	

0.058 ± 0.033 mg and 0.049 ± 0.029 mg for the 20's and 200's respectively; and comparisons between the second and the fourth week, the second and the sixth week, and the sixth and the eleventh week similarly showed no significance in the weight differences ($P = 0.3$ or more). The average ratio of food consumed/unit weight was 1.17 : 1 in favour of the 20's. This does not differ significantly from the average ratio of weights (1.15 ± 0.045) between the groups of larvae; however, the weight ratios of the larvae tended to increase significantly from the first to the thirteenth week, the extremes differing from the mean weight ratio of 1.15 ± 0.045 by a probability value $p = 0.01$, when the maximum deviation from the mean was assigned to the individual ratios; whereas there was no such trend in the food ratios (Table 2A). Similarly, when the weights of larvae in groups of 2 to 15, with no fewer than 10 in each category were compared, the differences in weight between the 2's and the 5's and the 2's and the 15's measured weekly between the fifth and eleventh weeks inclusive, became increasingly significant (Table 2B), whereas food consumption/unit weight of insect was generally not significantly different ($P = 0.3$ or more), notwithstanding an apparent upward trend (Table 2C).

Although the 20's gained increasingly and significantly in weight over the 200's, and the 15's over the 2's, the amounts of food consumed/unit weight were not

TABLE 2A—EFFECT OF FOOD CONSUMPTION ON GROWTH AND DEVELOPMENT OF LARVAE

Days	Wt. of food (mg/cockroach larva per day)			Wt. larva (mg)			Food consumed/unit wt. per day (mg)		
	20's	200's	Ratio	20's	200's	Ratio	20's	200's	
7	0.80	0.01	—	5.3	5.3	1.00	0.152	0.002	
14	1.07	0.58	1.85	11.3	10.6	1.09	0.092	0.055	
20	2.60	1.96	1.33	23.4	20.2	1.16	0.111	0.097	
27	5.40	4.44	1.22	45.2	39.3	1.15	0.119	0.112	
38	7.50	8.00	1.42	79.4	72.6	1.09	0.062	0.047	
42	2.44	3.60	0.67	112.0	104.0	1.08	0.022	0.035	
49	12.10	6.80	1.78	180.0	158.0	1.14	0.067	0.043	
52	12.10	4.06	3.00	195.8	176.0	1.11	0.062	0.023	
56	17.10	14.50	1.17	245.0	215.0	1.14	0.070	0.067	
63	11.70	6.30	1.86	328.0	285.0	1.15	0.036	0.022	
70	36.40	32.50	1.11	453.0	407.0	1.11	0.080	0.080	
77	21.00	10.74	1.96	588.0	483.0	1.22	0.036	0.022	
84	37.80	33.70	1.13	730.0	604.0	1.20	0.052	0.056	
91	15.30	14.95	1.00	817.0	614.0	1.23	0.019	0.019	
Av. 14.3 ± 12.6				Av. $1.15 \pm 0.045^*$			0.058 ± 0.033	$0.049 \pm 0.029^\dagger$	
			10.5 ± 9.5						

* The extremes differ from the mean significantly ($P = 0.01$).† $P = 0.3$.

TABLE 2B—NUMBER IN GROUP AND AVERAGE WEIGHT OF LARVAE (mg)

Days	2's-10	5's-14	10's-10	15's-14	<i>P</i> (2's vs. 15's)	<i>P</i> (2's vs. 5's)
35	91 ± 12	102 ± 17	95 ± 25	98 ± 24	0.5	0.1
42	137 ± 17	138 ± 28	136 ± 40	159 ± 31	N.S.	N.S.
49	206 ± 25	239 ± 47	230 ± 55	246 ± 56	<0.2	<0.1
57	269 ± 55	328 ± 86	330 ± 90	355 ± 88	0.5	0.2
64	438 ± 43	496 ± 80	475 ± 102	514 ± 98	0.1	<0.2
71	487 ± 86	589 ± 136	589 ± 130	663 ± 155	<0.05	<0.2
78	609 ± 105	791 ± 134	767 ± 138	845 ± 173	0.01 approx.	<0.02

N.S. = not significant.

TABLE 2C—WEIGHT OF FOOD CONSUMED BY SMALL GROUPS OF LARVAE, PER UNIT (mg) WEIGHT

Days	2's	5's	10's	15's
42	0.365	0.290	0.272	0.302
49	0.233	0.457	0.544	0.606
64	0.438	0.415	0.487	0.502
71	0.200	0.129	0.118	0.183
78	0.283	0.432	0.448	0.428
Av.	0.300 ± 0.090*	0.345 ± 0.137	0.374 ± 0.175	0.404 ± 0.166

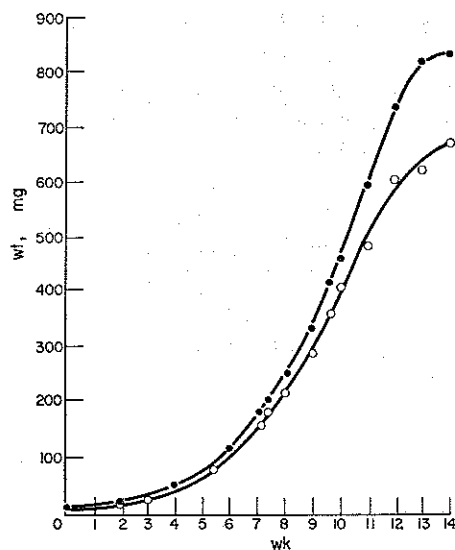
* *P*(2's vs. 15's) = 0.3 approx.

FIG. 1. Growth of larvae in groups of 20 (●) and 200 (○) showing increasing divergence of curves. See Table 2A for comparison of ratios of growth and food consumption.

significantly different in the various groups when compared at different times during larval development (Table 2A). It therefore seems probable that the larvae attained optimal growth because of a more efficient utilization of food rather than because of greater food intake.

'Crowding' leads to injury and cannibalism and thus creates a state of apprehension in the colony which causes a decline in growth rate by upsetting the metabolism and food intake (WHARTON *et al.*, 1967). However, there are other aspects of grouping which may affect growth. For example, contact or odorous pheromones may have a depressing effect, or the activity of stimulatory pheromones may be suppressed by another component in the animal's exudates or faeces, which may themselves also be toxic. The suboptimal growth shown by thinly populated groups could be due to the lack of stimulating contact, or to a too low concentration of a contact or air-borne pheromone, or to very complex influences. These and other possible growth factors will be examined in the following experiments.

Growth of larvae reared with adults

Isolated larvae grow more slowly than groups of larvae and at maturity weigh more than adults derived from groups of larvae (WHARTON *et al.*, 1967). By contrast, single larvae grown in the presence of adults (Table 3) grow at a faster rate than isolated larvae, and when they emerge the males weigh at least as much as males derived from isolated larvae; but females derived from single larvae which were reared in the presence of five mature mated or virgin females, or three males and three females, outweigh the females which mature from isolated larvae. Thus the single larva grows best in the presence of adults. It makes no difference whether the females are mated or not. The greater oöthecal production by the mated females could have supplied the larvae with a potent source of food, but the regular and frequent removal of the oöthecae minimized this source of error and there is no evidence that its influence was significant since the weights of both the developing larvae and adults in the two groups were similar and uncorrelated with the number of oöthecae eaten (Table 3). The adult influence thus appears to be exerted by both males and females. In accelerating the growth of the larva, adults act like groups of larvae, whereas in causing larger adults to be produced they are acting more like the isolated (single) larva. In other words, the larva becomes a large adult by being separated from other larvae. The larvae went through their entire period of development with a low mortality, or without any evidence of having been attacked by the adults even when males were present. The few deaths that occurred resulted from injury incurred during the frequent inspection and rearrangement of the jars while searching for oöthecae, checking on the presence of the small larvae, feeding, and so forth.

These distinctions further characterize the difference in growth habits between *P. americana* and *B. germanica* in that the latter, according to CHAUVIN (1946), not only grows best in isolation (except for groups of 5), but is unaffected in its growth by the presence of adults.

TABLE 3.—INFLUENCE OF ADULTS¹ ON GROWTH OF LARVAE

Single larvae reared with										
5 virgin females*			5 mated females†			3 males and 3 females			5 males	
resultant adults			resultant adults			resultant adults			resultant adults	
Sex	M	F	M	F	M	M	F	M	F	M
No.	5	6	5	7	6	6	7	4	4	9
Av. weight (mg)	919 ± 126	1247 ± 236	1035 ± 81	1154 ± 170	893 ± 51	1230 ± 175	866 ± 48	928 ± 64	1004 ± 69	148 ± 24
Development time (days)	117 ± 5	111 ± 14	112 ± 0	103 ± 7	—	—	—	119 ± 11	133 ± 17	133 ± 17
Group of 15 reared with										
2 males and 4 females										
resultant adults			resultant adults			resultant adults			resultant adults	
No.	7	2	7	2	32	36	772 ± 78	970 ± 76	108 ± 11	100 ± 11
Weight (mg)	866†	1150†	866†	1150†	772 ± 78	970 ± 76	108 ± 11	100 ± 11	100 ± 11	100 ± 11
Development time (days)	117 ± 4.9	102 ± 12	117 ± 4.9	102 ± 12	108 ± 11	100 ± 11	100 ± 11	100 ± 11	100 ± 11	100 ± 11

* No. of oöthecae produced and eaten: 66/190

† No. of oöthecae produced and eaten: 165/911.

‡ Weighed as a group.

There is evidently more than one growth factor or, if only one, it is effective at different times and in different ways. For example, the group effect which accelerates the rate of growth of larvae so as to give much heavier 10's than 1's at 9 weeks of age, and hastens maturity, does not thereby give a heavier adult. But the action of adults on the larva, like that of groups of larvae, hastens maturity, and in addition gives adults as large as those from isolates.

Development

The development times of these several groups of larvae varied greatly and generally inversely with the weights, the isolates taking longest to mature and being slowest to gain weight (WHARTON *et al.*, 1967). The adults derived from isolated larvae weighed more than adults derived from groups of larvae, and in all conditions of density tested there was a tendency for the last adults that emerged to be the heaviest of their group (WHARTON *et al.*, 1967), so that there was a correlation between the development times of the larvae and the weights of the resultant adults. However, we have seen (Table 3) that adults derived from single larvae, or from a small group of larvae reared among adults, weigh as much as adults derived from isolated larvae even though they mature much faster than isolates, whereas under very crowded conditions, as with groups of 1000 (WHARTON *et al.*, 1967), larvae take much longer to mature than in less dense groups but nevertheless produce adults of lighter weight. It follows that development time cannot be correlated with adult weight in all cases.

Secretion from tergites

Larvae of *P. americana* secrete from their terminal segments copious amounts of a sticky exudate which ROTH and STAHL (1956) have shown consists largely of protein. Although larvae eat this material from one another, its function is unknown. As it is ingested and might therefore possibly be a growth regulator, fifteen larvae were isolated in 250 ml beakers and each fed the freshly collected exudate from a larva in the fifth or sixth instar. They were fed twice a day three times weekly, first by smearing the food pellet, and then, after about 6 weeks, by feeding the freshly gathered exudate directly to the insect with a glass rod. This procedure was continued for about 3 months, and finally the material was smeared over the body of the larva to permit its absorption through the cuticle. The larvae were little benefited if at all by feeding and smearing of the tergal exudate, the emergent males and females weighing 725 ± 96 mg and 926 ± 132 mg respectively. It is recognized, however, that in the natural state larvae may feed upon or come in contact with the exudate more frequently and that this might have a beneficial effect that artificial manipulation tends to obstruct.

We have seen some specimens that were extremely stunted in growth and slow in development, presumably because of the lack of some intrinsic growth factor. Such larvae might still be in their third or fourth instar when all the others have matured, their growth not even comparable to that of the 1's. On two occasions we have had them as one of a group of two larvae which had been put together

immediately after hatching, and in each the companion larva grew at the normal rate for groups of 2 and attained a size equal to that of adults from isolates. It is evident that the ability of the companion larva to grow normally in the presence of a runt—that is, as it would in the presence of any other larva, and therefore faster than a single, isolated larva—indicates that it received a growth stimulus from the runt even though the latter lacked or was deficient in (a) an intrinsic growth factor, or/and (b) the capacity to respond to the stimulus of its companion; or that the normally growing larva itself lacked the capacity to promote growth in the runt. The last idea is hardly tenable, however, since runts have been seen among large groups of normally growing larvae. Therefore the lack of an intrinsic growth factor, or of the capacity to respond to the stimulus of their companions, seemingly in no way interfered with the ability of the runts to stimulate their companions to normal growth. The adult size attained by the companion larva suggests that the runt may lack the capacity to restrain adult growth which groups of larvae apparently possess. Certainly, the exudate of the cerci plus terminal segments of these minute larvae is trifling in amount and, in view of the at best weakly positive results of our feeding experiments with the exudate of normal larvae, too little to effect a significant increase in the growth rate of other larvae—if growth promotion is indeed one of its functions. No air-borne pheromone seems plausible, in view of our negative results with an air current passed over groups of larvae onto other, isolated larvae (see below).

Shelter

Larvae grown in 6 × 8 in. jars with a shelter consisting of an inverted paperboard pint container were shown to have grown at a faster rate by 5 weeks of age than larvae without shelter (WHARTON *et al.*, 1967). Tables 4 and 5 show that this disparity continues throughout the development of both isolated and grouped larvae, with a highly significant difference in weights ($P = 0.01$) and in food consumption in favour of the sheltered larvae. Notwithstanding the fact that this difference is maintained even late in larval development, the adults that emerge do not differ significantly in weight, sex for sex.

To determine whether the paper cartons contain a growth-promoting chemical, cartons were extracted with ether and then with water, and other cartons were pulped in water and the supernatant extract decanted. Cartons made of Whatman No. 1 filter paper were each impregnated with a different one of the three extracts and then dried at room temperature. The cartons were then placed in 6 × 8 in. battery jars containing twenty newly hatched larvae. Control jars were set up with (a) Whatman-paper cartons impregnated with aqueous extracts of cartons which had been previously extracted with ether, or (b) untreated Whatman paper, or (c) without a cylinder of any kind. The jars were incubated in the dark at $29 \pm 1^\circ\text{C}$ and the larvae weighed at intervals. Except for the insects whose shelter had been impregnated, there was no significant difference in time of development or adult weight, but the males whose shelter had been treated with the ether extract weighed significantly less ($P = 0.02$) than those with untreated shelters, indicating

that the ether extract contained a growth depressant. That this effect is not apparent in intact cartons is probably due to the wax with which the cartons are impregnated and coated, which minimizes the effect, for isolates grown in jars with shelters of polypropylene cartons weighed appreciably more than isolates in the

TABLE 4—EFFECT OF SHELTER ON GROWTH AND DEVELOPMENT

Weeks	Groups of 10		<i>P</i>	Isolates		<i>P</i>
	With shelter wt. (mg)	Without shelter wt. (mg)		With shelter wt. (mg)	Without shelter wt. (mg)	
5	89.1 ± 5.2	67.4 ± 6	0.01	66 ± 19	44 ± 14	0.01
7	185	133 ± 7.6		106 ± 32	67 ± 27	
9	349 ± 52	237 ± 125		196 ± 65	105 ± 41	
11	529	383				
12	659 ± 66	515 ± 22	<0.01			
13				553 ± 203	254 ± 103	<0.01
15				673 ± 234	388 ± 169	0.015
No. and wt. (mg) of adults						
Males	32—772 ± 78	16—772 ± 85		6—922 ± 60	3—910 ± 37	
Females	36—970 ± 76	13—927 ± 107		7—1017 ± 69	6—1068 ± 138	
No. and development time (days)						
Males	15—126 ± 23	16—121.4 ± 15.4		6—147.5 ± 23.5	3—170.3 ± 21	
Females	14—106.2 ± 16.2	13—117.5 ± 15.1		7—133.3 ± 17.4	6—143.3 ± 14.2	

TABLE 5—EFFECT OF SHELTER ON FOOD CONSUMPTION (mg/week per larva)

Week	Groups of 10		Isolates	
	With shelter	Without shelter	With shelter	Without shelter
7-9	54.0	37.0	38.7	19.2
9-11	86.7	66.2	70.7	46.7
11-13	131.0	91.4		
13-14	238.7	203.3		
15-18			109.5 ± 36.1	83.7 ± 15.8

paper cartons, attaining weights of 1169 ± 138 mg and 1207 ± 134 mg for the males and females, respectively. It is evident that the paper cartons do not provide the best shelter for growth, but that they are better than no cartons.

It is interesting that we found (WHARTON and WHARTON, unpublished data) that a certain grade of brown wrapping paper was highly toxic for the insects. When placed as a cover over trays of beakers containing adult cockroaches, it proved to be lethal in 24-48 hr. Such papers often contain residues of chemicals used in their preparation, and urea formaldehyde, used in their bonding, may emit formaldehyde when wet.

Vision

Although the incubator in which the larvae were kept was lighted only briefly for routine care, it seemed desirable to determine the possible effect of light and vision on growth. Isolated larvae were placed in rectangular beakers made of 2 x 3 in. mirrors or plain glass and supplied with food and water. The mirrored and plain beakers were set alternately about 2 in. apart in the incubator under a constant light. The multiple images failed to stimulate growth in the isolated larvae as indicated by the fact that after 38 days there was no significant difference in the weights of the two sets.

TABLE 6—EFFECT OF CONSUMPTION OF FAECES ON GROWTH OF LARVAE

	Faeces fed		Faeces not fed	
	7 males	7 females	8 males	6 females
Resultant adults				
Wt. of adults (mg)	730 ± 102	900 ± 208	774 ± 74	952 ± 77
Development time (days)*	640 ± 118		641 ± 120	

* Larvae were maintained in 250 ml beakers at room temperature; hence slow rate of development (see WHARTON *et al.*, 1967).

Asepsis

Asepsis has been shown to depress the growth of certain insects (CLEVELAND *et al.*, 1934; GIER, 1947; HOUSE, 1949; BROOKS, 1954), while incorporation of 5 per cent faeces in the food was found to promote the growth of *B. germanica* (CHAUVIN, 1946). As isolation at hatching might be conducive to asepsis, it seemed that a lack of the full complement of the normal intestinal microbia might be a factor in inhibiting the growth of the isolated larvae. It therefore seemed desirable to supplement the PLC diet of the newly hatched larvae with faeces. Therefore isolated larvae were allowed free access to fresh faeces from adults which were placed on the floor of the beaker or smeared over the food pellet which constituted their normal diet. The larvae were observed to feed upon the faeces and were weighed on emergence as adults. Neither in time of development nor weight did the adults differ significantly from controls (Table 6). As the faeces were not incorporated in the food, we have no measure of the amount consumed, whereas CHAUVIN (1946) found that in contrast to the beneficial effect of 5 per cent, 10 per cent faeces was toxic. We cannot say that quantitative feeding would not reveal a beneficial dose, but our larvae must have sampled a wide range of doses and none seemed to gain or to be hurt by it.

The fact that at any stage grouping enhances while isolation retards the growth rate demonstrates conclusively that the influence is transient, and therefore argues against asepsis being a factor in the current experiments.

Cuticular exudates

On the possibility that exudates given in sufficient concentration might promote growth, six hundred instar larvae were extracted by gentle shaking for about 2 min in several hundred ml of 10% ethyl alcohol in isopentane. The colourless extract consisted of two phases and after being concentrated was pale yellow-brown in colour. Alcohol was added to the alcohol-lipid phase (GILBY and Cox, 1963) to bring its volume to 30 ml (Solution 1). The addition of alcohol to the aqueous phase resulted in a flocculent precipitate, probably from ejaculated faeces, and the further addition of ether made a single-phase pale-brown solution (Solution 2) and precipitated the fluffy material in lumps. Small plastic bottle caps perforated with nine to ten small holes around the periphery about $\frac{1}{8}$ in. from the top, to permit ventilation but to exclude the larvae, and each containing a shallow glass dish, were sealed with Mylar covers having a small hole at the top to permit injection with a 26 gauge hypodermic needle. The sealed caps were placed in 250 ml beakers, each of which contained one newly hatched larva and a supply of food and water. On alternate days, except week-ends, the sealed caps were removed from the beakers and in half of them 0.05 ml of Solution 1 was injected through the caps into the dishes of those beakers containing the control larvae which were fed PLC only. The solvent was allowed to evaporate and the caps then replaced in the beakers. The larvae which had been fed extract weighed 21.0 ± 5.0 mg and the controls 17.4 ± 4.9 mg after being treated for 5 weeks, which is probably not significantly different ($P > 0.1$). Larvae from the same stock were allowed to walk and feed upon the dried residue of 0.5 ml of Solution 1 placed in uncovered dishes. Thirty-nine days later these larvae weighed 15.8 ± 7.0 mg, which was not significantly ($P = 0.5$; $P = 0.1$) less than controls fed PLC only or those exposed to the odour alone. The aqueous phase was shown to be no more effective in promoting growth, the weights of fed and control larvae after 26 days being 14.6 mg and 15.6 mg, respectively.

Taken together with the observation that growth was not promoted by feeding freshly killed larvae to other larvae supplied with an adequate diet (WHARTON *et al.*, 1967), these findings indicate that larvae of the American cockroach do not produce significant amounts of a growth-promoting contact pheromone.

Air-borne pheromone

To determine whether or not an air-borne growth-promoting pheromone caused increased growth among grouped cockroach larvae, two sets of 1.5 l. glass jars were fitted with rubber stoppers having an inlet and an outlet glass tube. The inlet tube led to the bottom of the jar, and the outlet tube, opening near the stopper, to a train of ten 200 ml rubber-stoppered jars connected by glass tubing. The jars were all supplied with PLC pellets and water, and were not vaselined. Two hundred and fifty newly hatched larvae were placed in one of the 1.5 l. jars and single newly hatched larvae in each of the 200 ml jars. The second 1.5 l. jar contained no larvae but was connected to its own train of isolated larvae which served as controls. A

stream of air flowing at the rate of 250 ml/min was passed through each system of jars, in which the temperature was 79 to 82°F and the r.h. 50 to 70%. The air passed over the larvae in the 1.5 l. jar is referred to as the 'roach stream', and that passed through the empty jar simply as the 'air stream'.

After 73 days the larvae in the 'roach stream' did not weigh significantly more than those in the 'air stream', while the two hundred and fifty larvae at the source averaged only 71.1 ± 34 mg, even though survival conditions were such that only nine had died. As the low weight of the larvae at the source suggested that growth

TABLE 7—EFFECT OF VAPOURS FROM GROUPS OF LARVAE ON GROWTH OF ISOLATED LARVAE

Time (days)	Source 250 larvae wt. (mg)	Isolates		
		In 'roach stream' wt. (mg)	In 'air stream' wt. (mg)	
13	22.7	22.9 ± 9.3	16.1 ± 5.6	$P = 0.08$
73	71.1 ± 34 (30 larvae)	45.2 ± 12.1	37.5 ± 17.9	$P = 0.25$
	Source 20 larvae (from above)			
102	268.7 ± 103	75.0 ± 33	58.1 ± 24.6	$P = 0.25$
119	434.4 ± 100	73.0 ± 32	61.0 ± 22	$P = 0.4$

inhibitors might be present which would enter the 'roach stream' and inhibit growth of the isolates, the larvae were removed from the source jar, the jar was cleaned, and only twenty of the larvae replaced, and the air flow then resumed. With twenty larvae in the jar, conditions for growth approached optimal (WHARTON *et al.*, 1967) and, moreover, as the air in the source jar was changed every 4 min the flow was considered sufficient to transmit compounds of even relatively high density through the train. The grouped larvae did in fact grow much faster than the isolates (Table 7), so that it could be presumed that conditions were suitable for producing and transmitting by air any volatile pheromone that the larvae might have produced; nevertheless, weights taken 29 and 46 days later showed no significant difference ($P = 0.4$) between the two sets of isolates. Failure of the larvae exposed to the 'roach stream' to grow significantly faster than the controls in the 'air stream' is evidence that little, if any, air-borne growth pheromone is produced by the *P. americana* larva.

Contact and display

In males of the desert locust, the transition from immaturity to maturity, is initiated by contact with other males. This may be actual or, less effectively, proximal and involves the transfer of a pheromone which can be carried by contact or by air (NORRIS, 1954; LÖHER, 1961). In our studies with larvae of *P. americana*

no such pheromone could be demonstrated. However, in view of our generally negative results with other environmental factors that might affect growth, contact in its broader sense deserves to be considered, especially as CHAUVIN (1946) has concluded that antennal contact depresses the growth of larvae of *B. germanica*.

The behaviour of cockroach larvae, which is pertinent to growth, has been little studied, whereas much is known about the behaviour of adults (RAU, 1940; ROTH and WILLIS, 1952; WHARTON *et al.*, 1954a, b; BARTH, 1964; EWING, 1967). Contact may be considered as due to chance, or as a formal expression of association. The probability of chance contact increases with the density of population, and beyond a certain point of congestion results in injury, increased mortality, and retarded growth (WHARTON *et al.*, 1967). On the other hand, formal contact can be expressed more freely in less crowded conditions and is observed most fully in groups of only a few insects. The isolated insect by contrast lives in a circumscribed world of stimuli limited to its needs of subsistence, is denied the contacts available to groups, and is slow in growth.

When two larvae which have been isolated are placed in a clean beaker, they perform a kind of dance involving display and contact which may be aggressive or gentle. The insects at first stand tensely diametrically apart, turn their heads slightly towards one another, and scan the air with their antennae. One then moves along the periphery of the beaker in slow, brief, widely spaced measures as if scouting the situation, and the other unpredictably becomes startled and jumps away. There is usually no contact and the insects become seemingly indifferent to each other. They palpate the substrate, salivate, and move about the beaker. On coming to the spot where each had stood they sweep their antennae through each other's saliva and pass them across their mouth-parts, while their anterior palpi become highly agitated but apparently without touching the antennae. The animals become more tense and active and, facing head on, enter a brief passage of antennal fencing, which at first consists of a vigorous waving without contact. This is followed by more intense activity in which the antennae cross those of their opponent at different angles, and if contact is made it is seemingly at the midsection of their lengths. After this, one or both of the larvae will stand high on their legs in a stilted manner, with abdomens bent upwards, and raise a rear leg high and laterally so as to tilt their bodies toward each other at a distance of 2 or more cm. This tense posture may be held for many seconds or for as long as a minute and then relaxed slowly as the leg descends, but it may be resumed even before the leg touches the floor. Unless the insects then engage one another, one will walk away with a stilted gait and its cell-mate then approach the spot and palpate the area where it had been. The insects approach one another in a sidling, seemingly menacing manner facing in opposite directions. They may strike one another with their legs or bite and pull them, and may then separate and resume the earlier abdomen- and leg-lifting ritual, the smearing of the palpi and antennae, and the antennal exchange. After a few episodes of this kind, the animals become quieter, and from a distance just within reach each may extend a leg gently, tentatively, toward the other, laying its foot upon it or plying it with light taps. The

recipient of these attentions seldom shrinks from them, and the extended leg may tug at the recipient's to draw it toward itself.

If a third insect is introduced into a beaker containing two larvae, it may be pursued and attacked by one or both of them with leg biting and tugging. This may go on for some time and then the ritual of accommodation will begin with one of the two original occupants, while the other stands by. There may be sporadic aggression and less certain accommodation, as may be seen occasionally even in large groups of larvae that have been together since hatching. In time, the larvae will be found in a huddle, so characteristic of the cockroach.

Aggressive behaviour is by no means the usual result of an encounter between larvae or even adults of *P. americana*. Some of the tentative or cautious attitudes described may be assumed by the larvae at first meeting, but even when a food pellet in the domicile of one of the cockroaches is the bone of contention, which the 'owner' characteristically rides while keeping the 'invader' at a lower level, hostile behaviour is seldom pursued to the point of outright conflict. Antagonistic behaviour might occur in crowds or where food is short, but between two cockroaches it usually ends in a few minutes or hours with a degree of acceptance that finds the 'invader' ensconced perhaps on the food pellet, while the 'owner' rests elsewhere. Adults behave similarly. However, when nubile females are present or a choice morsel of food is put before them, males may fight ferociously and maim one another, but they seldom fight to the death, nor is death the usual consequence of aggression as it is in *Nauphoeta cinerea* (Oliv.) (EWING, 1967).

There are thus many modes of contact among groups of larvae which result in forms and degrees of accommodation that stamp the relationships of members of a colony. The encounter of previously isolated larvae is inevitably diverting and stimulating and serves to reinforce the sense of security and well-being which a gregarious animal finds among others of its kind, and must therefore be conducive to the activation of metabolic functions favouring growth. Since grouped larvae in fact develop at a faster rate than isolated ones it is evident that antennal contact has not inhibited growth in the American cockroach as it is said to do in the German cockroach (CHAUVIN, 1946), but rather may have promoted it.

The remarkable facts that isolation of larvae from groups results in a prompt retardation of growth, and the formation into groups of larvae that had been isolated results in a prompt increase of growth (WHARTON *et al.*, 1967), indicate that the principal stimulus to growth depends on the physical presence of other larvae and is of short duration and immediate. Physical contact has been largely responsible for activating other modes of awareness. But while only one or a few of these may suffice at any given time to secure the social bond and direct its power, the influence is transient, and the stimulus of physical contact continually needs to be renewed.

CONCLUSIONS

Reviewing the various experimental findings, we note the salient fact that of all the growth-initiating factors considered the isolated larva lacks one, and that is

contact. Without contact with other larvae it grows slowly but is nevertheless able to develop into a large adult. Groups of larvae, on the other hand, make contact with one another freely and grow and mature more rapidly apparently because of this, since neither an air-borne pheromone(s) nor body exudates promoted growth, but the adults which emerge from groups of larvae are smaller than those from isolates. Single larvae reared with adults are also subject to contact and grow and develop as rapidly as grouped larvae, but they nevertheless develop into larger adults. Contact then is evidently a principal exteroceptive stimulus which promotes growth and development of the larva.

Assuming that heavier adults derived from isolated larvae implies that *at each ecdysis* isolated larvae are heavier than those in groups, though being slower in development they are lighter at any given time, then ecdysone could be considered as a dominant growth factor which is activated or suppressed by the exteroceptive stimuli of grouping and responsible for much of the weight difference observed, and indirectly for the rate of development. Ecdysone depresses the motor neurones (HASKELL and MOORHOUSE, 1963) and stimulates ribonucleoprotein synthesis and growth in muscle (WIGGLESWORTH, 1963). In locusts it is more abundant in isolated than in grouped insects (ELLIS and CARLISLE, 1961; CARLISLE and ELLIS, 1963) and disappears after the adult ecdysis except in the isolated insect, in which it persists. Its persistence in the isolated *P. americana* larva through the last instar to the adult could account for the gain in weight, whereas its regression in groups of larvae could explain their lesser weight. Ecdysone could be indirectly responsible for the slower *rate* of growth of the isolated insect in that its depression of the motor neurones would cause reduced locomotor activity, as we have observed in isolates (WHARTON and WHARTON, unpublished data), and as a consequence to a relatively lower metabolic rate.

REFERENCES

- ALLEE W. C. (1934) Recent studies in mass physiology. *Biol. Rev.* **9**, 1-48.
- BARTH R. H., JR. (1964) The mating behaviour of *Byrsotria fumigata* (Guérin) (Blattidae, Blaberinae). *Behaviour* **23**, 1-30.
- BROOKS M. A. (1954) Certain aspects of the histochemistry and metabolic significance of the intracellular bodies (bacteroids) of cockroaches (Blattariae). Ph.D. Thesis, University of Minnesota.
- CARLISLE D. B. and ELLIS P. E. (1963) Prothoracic gland and gregarious behaviour in locusts. *Nature, Lond.* **200**, 603-604.
- CHAUVIN R. (1941) Contribution à l'étude physiologique du criquet pèlerin et du déterminisme des phénomènes grégaires. *Bull. Soc. ent. Fr.* **110**, 133-272.
- CHAUVIN R. (1946) Notes sur la physiologie comparée des Orthoptères—V. L'effet de groupe et la croissance larvaire des blattes, des grillons et du Phanéroptère. *Bull. Soc. zool. Fr.* **71**, 39-48.
- CLEVELAND L. R., HALL S. R., SANDERS E. P., and COLLIER J. (1934) The wood-feeding roach *Cryptocercus*, its protozoa, and the symbiosis between protozoa and roach. *Mem. Am. Acad. Arts Sci.* **17**, 185-342.
- ELLIS P. E. and CARLISLE D. B. (1961) The prothoracic gland and colour change in locusts. *Nature, Lond.* **190**, 368-369.
- EWING L. S. (1967) Fighting and death from stress in a cockroach. *Science, N.Y.* **155**, 1035-1036.

- GIER H. T. (1947) Growth rate in the cockroach *Periplaneta americana* (Linn.). *Ann. ent. Soc. Am.* **40**, 303-317.
- GILBY A. R. and COX M. E. (1963) The cuticular lipids of the cockroach, *Periplaneta americana* (L.). *J. Insect Physiol.* **9**, 671-681.
- HASKELL P. T. and MOORHOUSE J. E. (1963) A blood-borne factor influencing the activity of the central nervous system of the desert locust. *Nature, Lond.* **197**, 56-58.
- HOUSE H. L. (1949) Nutritional studies with *Blattella germanica* (L.) reared under aseptic conditions—II. A chemically defined diet. *Can. Ent.* **81**, 105-112.
- LANDOWSKI J. (1937) The influence of isolation and cohabitation on the development and growth of nymphs of *Periplaneta orientalis* L. *C. R. Soc. Sci. Lett. Warsaw* **4**, 190-203 (translated from the Polish).
- LEFKOVITCH L. P. (1964) The growth of restricted populations of *Lasioderma serricorne* (F.) (Coleoptera, Anobiidae). *Bull. ent. Res.* **55**, 87-96.
- LÖHER, W. (1961) The chemical acceleration of the maturation process and its hormonal control in the male of the desert locust. *Proc. R. Soc. (B)* **153**, 380-397.
- MCFARLANE J. E. and HENNEBERRY G. O. (1965) Inhibition of the growth of an insect by fatty acids. *J. Insect Physiol.* **11**, 1247-1252.
- NORRIS M. J. (1954) Sexual maturation in the desert locust (*Schistocerca gregaria* Förskal) with special reference to the effects of grouping. *Anti-locust Bull.* **18**, 1-44.
- PETTIT L. C. (1940) The effect of isolation on growth in the cockroach *Blattella germanica*. *Ent. News* **51**, 253.
- RAU P. (1940) The life history of the American cockroach *Periplaneta americana* Linn. (Orthop.: Blattidae). *Ent. News* **51**, 273-278.
- ROTH L. M. and STAHL W. H. (1956) Tergal and cercal secretion of *Blatta orientalis* L. *Science, N.Y.* **123**, 798-799.
- ROTH L. M. and WILLIS E. R. (1952) A study of cockroach behavior. *Am. Mid. Nat.* **47**, 66-129.
- WHARTON D. R. A., LOLA J. E., and WHARTON M. L. (1967) Population density, survival, growth, and development of the American cockroach. *J. Insect Physiol.* **13**, 699-716.
- WHARTON D. R. A., MILLER G. L., and WHARTON M. L. (1954a) The odorous attractant of the American cockroach, *Periplaneta americana* (L.). *J. gen. Physiol.* **37**, 461-469.
- WHARTON D. R. A., MILLER G. L., and WHARTON M. L. (1954b) The odorous attractant of the American cockroach, *Periplaneta americana* (L.). *J. gen. Physiol.* **37**, 471-481.
- WHARTON M. L. and WHARTON D. R. A. (1957) The production of sex attractant substance and of oöthecae by the normal and irradiated American cockroach *Periplaneta americana* L. *J. Insect Physiol.* **1**, 229-239.
- WIGGLESWORTH V. B. (1963) The action of moulting hormone and juvenile hormone at the cellular level in *Rhodnius prolixus*. *J. exp. Biol.* **40**, 231-245.